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Yaba Monkey Tumor Virus Encodes a Functional Inhibitor of Interleukin-18▽

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Interleukin-18 (IL-18) is a critical proinflammatory cytokine whose extracellular bioactivity is regulated by a cellular IL-18 binding protein (IL-18BP). Many poxviruses have acquired variants of this IL-18BP gene, some of which have been shown to act as viral virulence factors. *Yaba monkey tumor virus* (YMTV) encodes a related family member, 14L, which is similar to the orthopoxvirus IL-18BPs. YMTV 14L was expressed from a baculovirus system and tested for its ability to bind and inhibit IL-18. We found that YMTV 14L bound both human IL-18 (hIL-18) and murine IL-18 with high affinity, at 4.1 nM and 6.5 nM, respectively. YMTV 14L was able to fully sequester hIL-18 but could only partially inhibit the biological activity of hIL-18 as measured by gamma interferon secretion from KG-1 cells. Additionally, 17 hIL-18 point mutants were tested by surface plasmon resonance for their ability to bind to YMTV 14L. Two clusters of hIL-18 surface residues were found to be important for the hIL-18–YMTV 14L interaction, in contrast to results for the *Variola virus* IL-18BP, which has been shown to primarily interact with a single cluster of three amino acids. The altered binding specificity of YMTV 14L most likely represents an adaptation resulting in increased fitness of the virus and affirms the plasticity of poxviral inhibitor domains that target cytokines like IL-18.

Interleukin-18 (IL-18) is a member of the IL-1 cytokine superfamily that plays a central role in inflammation and the development of the adaptive immune response (6). Initially described as gamma interferon (IFN-γ) stimulatory factor, IL-18 also induces other proinflammatory cytokines and chemokines (26), promotes NK cell activity (19), and helps drive the development of type 1 T cells (21). As a consequence, it is an important mediator in the host response to many viral and bacterial infections. Produced as an inactive precursor, it is cleaved by activated caspase 1 (also called IL-1-converting enzyme), to produce biologically active IL-18 that is then secreted (8). Extracellular IL-18 exerts its biological effects by binding a heterodimeric receptor complex composed of the ligand binding chain, IL-18 receptor alpha (IL-18Rα), and the signal-transducing chain, IL-18Rβ (4). Three clusters of surface residues of IL-18 were determined, by crystal structure and site-directed mutagenesis, to be critical for the initiation of proper signaling: sites I and II have been shown to be important for IL-18Rα binding, and site III is likely important for the IL-18–IL-18Rβ interaction (10).

The constitutive expression of IL-18 precursor from resting monocytes can be detected at both the mRNA and protein level, but the activation and secretion of bioactive IL-18 ligand are under further control by a host cell complex called the inflammasome (16, 17). A second level of IL-18 regulation occurs after the secretion of the cytokine. This extracellular control is mediated by an IL-18 binding protein (IL-18BP) that is a high-affinity (dissociation constant of 400 pM) IL-18 antagonist distinct from the cytokine receptors (15). At a twofold molar excess, this inhibitor can completely abolish the IFN-γ-inducing activity of IL-18 (25). Dysregulation of the cellular IL-18BP can lead to proinflammatory diseases, including systemic lupus erythematosus, rheumatoid arthritis, and Crohn's disease (5).

IL-18BPs have been identified and characterized in several poxviruses both in vitro and in vivo and are highly homologous to their mammalian counterparts (13, 14). The vaccinia virus IL-18BP (C12L) has been shown to promote virulence in a murine intranasal model (20). Additionally, the ectromelia virus IL-18BP (p13) has been shown to be important in downregulating the natural killer cell response in mice (1).

The exact nature of the human IL-18BP (hIL-18BP)–IL-18 interaction was explored by modeling the complex using the IL-1–IL-1R crystal structure and identified specific residues which may be involved in binding (11). Subsequent mutagenesis studies of hIL-18BP and *Molluscum contagiosum virus* (MOCV) IL-18BP (MC054L) supported this model and demonstrated the conservation of functional epitopes in mammalian and viral proteins (23, 24). A related study with *Variola virus* (VARV) IL-18BP has also been performed by mutagenesis of some of the surface residues of hIL-18. Three residues within site II on hIL-18 were found to be important for the binding of VARV IL-18BP (13).

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Yaba monkey tumor virus (YMTV) is a member of the *Yatapoxvirus* genus of poxviruses. This virus produces a very distinct disease in primates that is characterized by epidermal histiocytomas of the head and limbs (7, 12). Although the exact host reservoir of YMTV is not established, it is presumed that the immunomodulatory proteins expressed by this virus can at least partially cope with the primate/human immune system. Upon analysis of the YMTV genome (2), we found that this virus encoded a predicted IL-18BP family member, designated 14L. To test whether the 14L protein was indeed a functional inhibitor of IL-18, this protein was expressed and tested in vitro for its ability to bind and inhibit IL-18. We report that the YMTV 14L is able to bind both hIL-18 and murine IL-18 (mIL-18) with affinities in the low nanomolar range. While 14L is able to functionally sequester hIL-18, it can only partially inhibit the biological function of soluble hIL-18 ligand. We map the binding site on hIL-18 to a different region than the previously characterized VARV IL-18BP.

MATERIALS AND METHODS

Reagents. Recombinant human tumor necrosis factor (TNF), hIL-18, and mIL-18 were obtained from Biosource International. hIL-18BPa, soluble IL-18Rα, IL-18Rα blocking antibody, and neutralizing antibody to hIL-18 were purchased from R&D Systems. Protein A/G PLUS agarose was obtained from Santa Cruz Biotechnology. YMTV (VR587) was obtained from the American Type Culture Collection and grown on CV1 cells at 34°C.

Construction of recombinant baculovirus expressing YMTV 14L. 14L was PCR amplified from YMTV genomic DNA such that the native signal sequence was omitted. The signal sequence from myxoma virus T7 was also PCR amplified and was annealed to the 14L gene. The chimeric gene was cloned into pcDNA3.1 Myc/His (Invitrogen). Both a Myc/His-tagged and an untagged version were PCR amplified, using the pcDNA3.1 Myc/His construct as a template. These products were each cloned into pFastbac 1 (Invitrogen), and recombinant baculoviruses (AcY14L and AcY14L Myc/His) were produced by using a Bac-to-Bac system according to the manufacturer's protocol (Invitrogen). DNA sequencing was used to verify the inserted gene.

Production and purification of YMTV 14L. YMTV 14L Myc/His was produced by infecting High Five (Invitrogen) cells with baculovirus expressing 14L (AcY14L Myc/His [AcY14L-M/H]) in Express Five medium (Invitrogen) and incubating at 27°C for 72 h. The clarified supernatants were concentrated, and buffer was exchanged with 50 mM sodium phosphate, 500 mM sodium chloride, and 10 mM imidazole at pH 7.5. The supernatants were then purified by using TALON metal affinity resin (BD Bioscience) according to the manufacturer's protocol. The purified protein was concentrated and dialyzed against phosphate-buffered saline (Pierce). Untagged protein (AcY14L) purified by ion-exchange and size exclusion chromatography was kindly provided by Viron Therapeutics, Inc. All experiments, with the exception of the BIAcore analysis of the hIL-18 mutants, were performed with both tagged and untagged protein with no detectable differences in binding or activity.

Biomolecular interaction analysis using surface plasmon resonance (SPR). On a BIAcore2000 biosensor, either AcY14L, hIL-18BP, or soluble IL-18Rα was immobilized on a CM-5 BIAcore chip by using standard amine-coupling chemistry (9). The density of the protein was controlled such that the r_{max} was <120 relative units. hIL-18, mIL-18, and the hIL-18 mutants were injected at a flow rate of 50 μl/min in a volume of 100 μl at various concentrations. Once the injection was complete, HBS-P (BIAcore) was run over the chip for the dissociation phase. The chip surface was regenerated using 10 mM glycine, pH 1.5.

The sensograms were analyzed with BIAevaluation software (BIAcore). To correct for refractive index changes, sensograms from the control surface were subtracted from test protein sensograms. The binding data from each of the proteins were globally fitted to a 1:1 binding model. Experiments were performed several times with several different preparations of the 14L protein with similar results.

Inhibition of hIL-18-induced IFN-γ production. hIL-18 (10 ng/ml), TNF-α (10 ng/ml) (both from Biosource International), and various concentrations of purified 14L were incubated in a 96-well plate at 37°C for 30 min in complete RPMI medium. Human KG-1 cells were then added at a final concentration of 2×10^6 cells per ml and incubated for 24 h. After 24 h, the cultures were frozen and thawed three times, and the clarified supernatants were assayed for human IFN-γ by enzyme-linked immunosorbent assay (ELISA) (eBioscience).

Immunoprecipitation of hIL-18 with 14L. Protein A/G plus beads (Santa Cruz) were incubated with anti-penta-His monoclonal antibody (QIAGEN) for 1 h and washed with complete RPMI medium. Supernatant from cells infected with either AcY14L or *Autographa californica* nucleopolyhedrosis virus polyhedrin minus (AcNPVpolh$^-$; negative control) was then added, and the mixture was further incubated for 1 h. After being washed, the beads were mixed at various ratios. hIL-18 (100 ng/ml) was added to the beads and allowed to complex for 30 min. The clarified supernatants were added at a 1 in 10 dilution to KG-1 cells (2×10^6 cells per ml) in complete RPMI medium with 10 ng/ml of TNF and allowed to incubate at 37°C for 24 h. After 24 h, the cultures were frozen and thawed three times and the clarified supernatants were assayed for human IFN-γ by ELISA.

Production and purification of hIL-18 point mutants. hIL-18 was purified exactly as described previously (13). Briefly, constructs encoding wild-type and mutated hIL-18 were grown in BL21 cells (Novagen) and induced with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). The bacteria were lysed in B-PER (Pierce), and hIL-18 was bound to Ni-nitrilotriacetic acid resin (QIAGEN). hIL-18 was cleaved from the beads by using factor Xa (New England Biolabs) and subsequently purified by using a Superdex 75 column (GE Healthcare). The protein concentrations were determined by using a Bradford assay (Bio-Rad).

RESULTS

Kinetic analysis of IL-18 binding to purified YMTV 14L protein. SPR is a method that has been used to determine the detailed binding kinetics of several IL-18BPs (18, 25). To investigate the potential binding between the 14L protein and hIL-18, we first replaced the native signal sequence of Y14L with the signal sequence from M-T7, the efficiently secreted IFN-γ binding protein from myxoma virus, to facilitate the secretion of the 14L protein from a recombinant baculovirus vector (data not shown). Purified 14L was then immobilized to a CM5 chip by cross-linking primary amine residues to the dextran surface. The binding of both recombinant hIL-18 and mIL-18 to 14L was analyzed on a BIAcore2000 biosensor (Fig. 1). hIL-18 and mIL-18 bound with high affinity to 14L. The sensograms are characterized by a high on rate and a relatively low off rate (Fig. 1).

The sensogram data were globally fitted to a 1 to 1 binding model. Consistent with the affinities of other poxviral IL-18BPs, the affinity constants were calculated to be in the low nanomolar range, at 4.11 nM for hIL-18 and 6.47 nM for mIL-18 (Table 1).

Inhibition of IL-18 activity as monitored by IFN-γ secretion. Several studies have used the production of IFN-γ by KG-1 cells as a measure of the bioactivity of IL-18 (3, 25). We set out to examine the potential inhibitory properties of YMTV 14L by assaying the IL-18-dependent induction of IFN-γ from KG-1 cells. Similar to other IL-18BPs, YMTV 14L was able to inhibit the production of IFN-γ from KG-1 cells (Fig. 2). As more purified protein was added, a dose-dependent decrease in IFN-γ was observed. In contrast to other IL-18BPs, however, YMTV 14L was only able to inhibit the IFN-γ secretion by 50% at a 100-fold molar excess (Fig. 2). Shown are the results from a single experiment; however, multiple independent experiments using tagged and untagged versions of YMTV 14L protein confirm the result. Several control experiments were performed, including the addition of hIL-18BP, neutralizing antibody to hIL-18, and IL-18 receptor blocking antibody. All were able to fully inhibit IFN-γ production (data not shown), suggesting that a fraction of the IL-18 protein was in a state or conformation that was not functionally inhibited even when bound to 14L.

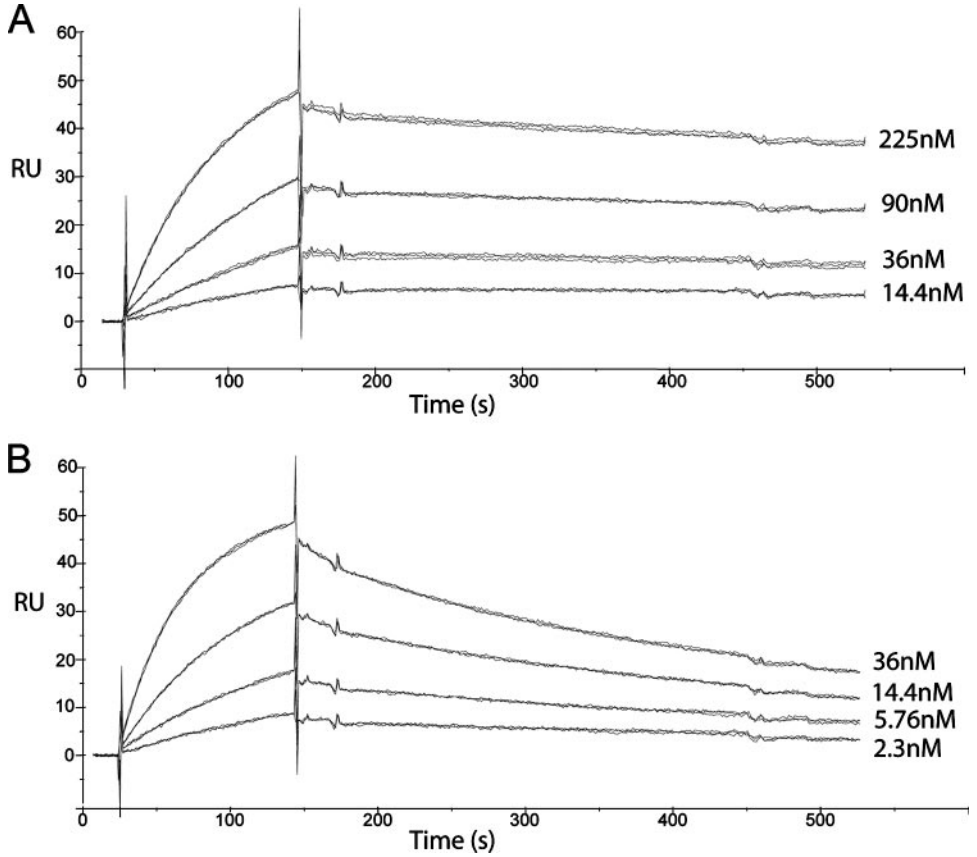

FIG. 1. YMTV 14L binds hIL-18 (A) and mIL-18 (B) with nanomolar affinity. YMTV 14L was immobilized to a CM5 chip and was analyzed on a BIAcore2000. (A) hIL-18 was injected over the chip at indicated concentrations for 120 s. (B) mIL-18 was injected over the chip at indicated concentrations for 120 s. Both sets of curves were globally fitted to a 1:1 binding model (BIAevaluation).

Sequestration of IL-18. Since YMTV 14L is unable to fully inhibit IFN-γ production in KG-1 cells, we set out to test whether the 14L protein is able to stably bind to biologically active hIL-18. To establish a sequestration assay for hIL-18, protein A/G beads were preabsorbed to an anti-penta-His antibody. The beads were then allowed to bind a C-terminally Myc/His-tagged version of the baculovirus-expressed YMTV 14L (AcY14L-M/H). These beads were mixed at various ratios (indicated in Fig. 3) with control beads lacking AcY14L-M/H. Each of these mixed bead samples was then incubated with hIL-18 and tested for the ability to sequester hIL-18. Following incubation and bead removal, the supernatant from each bead sample was tested for the presence of active hIL-18 by measuring IFN-γ induction from KG-1 cells. As increasing ratios of AcY14L-M/H loaded to control beads were allowed to interact with hIL-18, we observed a dose-dependent decrease in IFN-γ secretion (Fig. 3). In contrast to the results of the IFN-γ secretion activity assay, 14L was able to bind and sequester all of the biologically active hIL-18, thus confirming the SPR data showing that YMTV 14L is able to quantitatively bind and sequester all potential conformations of hIL-18 with high affinity.

TABLE 1. Kinetics and affinity constants of hIL-18 and mIL-18 binding to YMTV 14L[a]

Ligand	K_a (10^5/M · s)	K_d (10^{-4}/s)	K_D (nM)
hIL-18	1.1 ± 0.1	4.5 ± 0.7	4.11 ± 0.41
mIL-18	3.9 ± 0.1	25.5 ± 0.6	6.47 ± 0.18

[a] Values are the means ± standard deviations of the results. K_a, association rate constant; K_d, dissociation rate constant; K_D, dissociation rate.

The hIL-18 binding sites of YMTV 14L, hIL-18BP, and hIL-18Rα overlap. In order to verify that YMTV 14L can indeed interfere with IL-18 binding to its receptor, a competition experiment was designed. AcY14L was immobilized to a CM5 chip. Solutions containing 100 nM hIL-18 preincubated with various concentrations of either hIL-18BP or soluble hIL-18Rα were injected over the sensor chip surface (Fig. 4). A dose-dependent decrease in the binding of hIL-18 to YMTV 14L is observed when hIL-18 is complexed to the hIL-18BP or the soluble IL-18 receptor (Fig. 4). The reverse experiment, with either the hIL-18BP or the soluble IL-18Rα immobilized to the chip, showed the same result (data not shown).

Mapping the binding site on hIL-18. Since it is possible that 14L binds to IL-18 differently than other IL-18BPs, the binding site on hIL-18 was mapped. This was tested by using SPR for binding 14L against a panel of hIL-18 point mutants (13). The wild-type hIL-18, produced in bacterial vectors, bound to immobilized AcY14L with a higher affinity than did commercial hIL-18, and so the comparisons were all made with identically

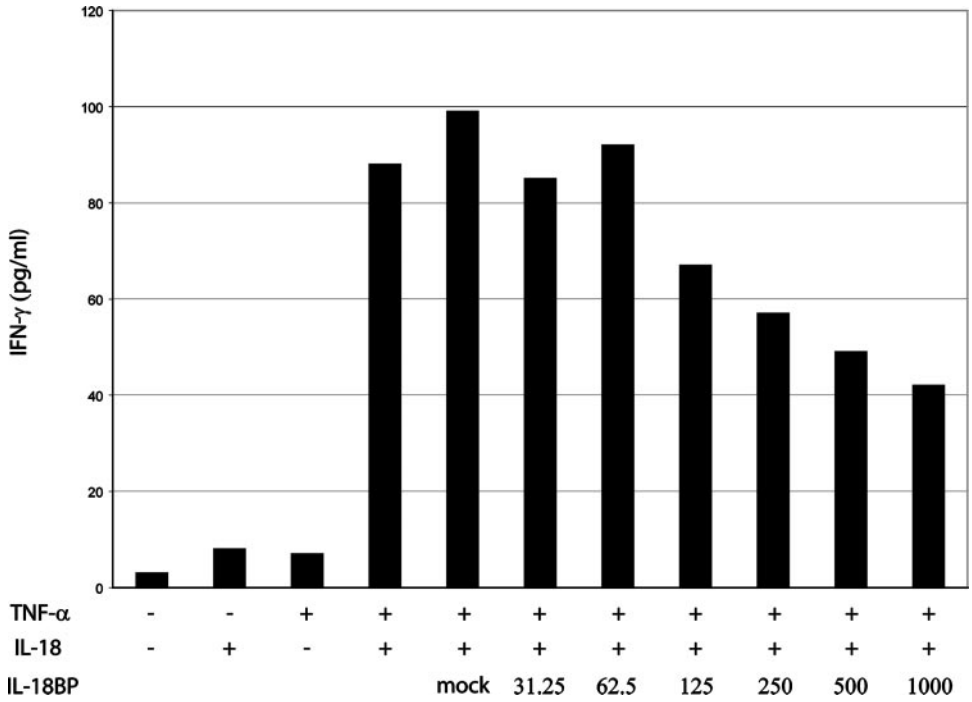

FIG. 2. Production of IFN-γ is inhibited by AcY14L. AcY14L was added at various concentrations (nanograms/milliliter) to wells containing TNF-α (5 ng/ml) and IL-18 (10 ng/ml). KG-1 cells were added, and 24 h later, IFN-γ was assayed in duplicate by ELISA. +, present; −, absent.

produced hIL-18 mutants expressed in the identical fashion from IPTG-induced bacteria. Compared to the affinity of the wild-type hIL-18, several alanine substitution mutants exhibited a lower affinity with 14L protein (Table 2). These hIL-18 point mutations can be separated into two distinct groups: those involving amino acids that are in site I and those involving residues that are in site II. Those substitutions that have the greatest effect on affinity (L5A, K53A, S55A, R58A, and

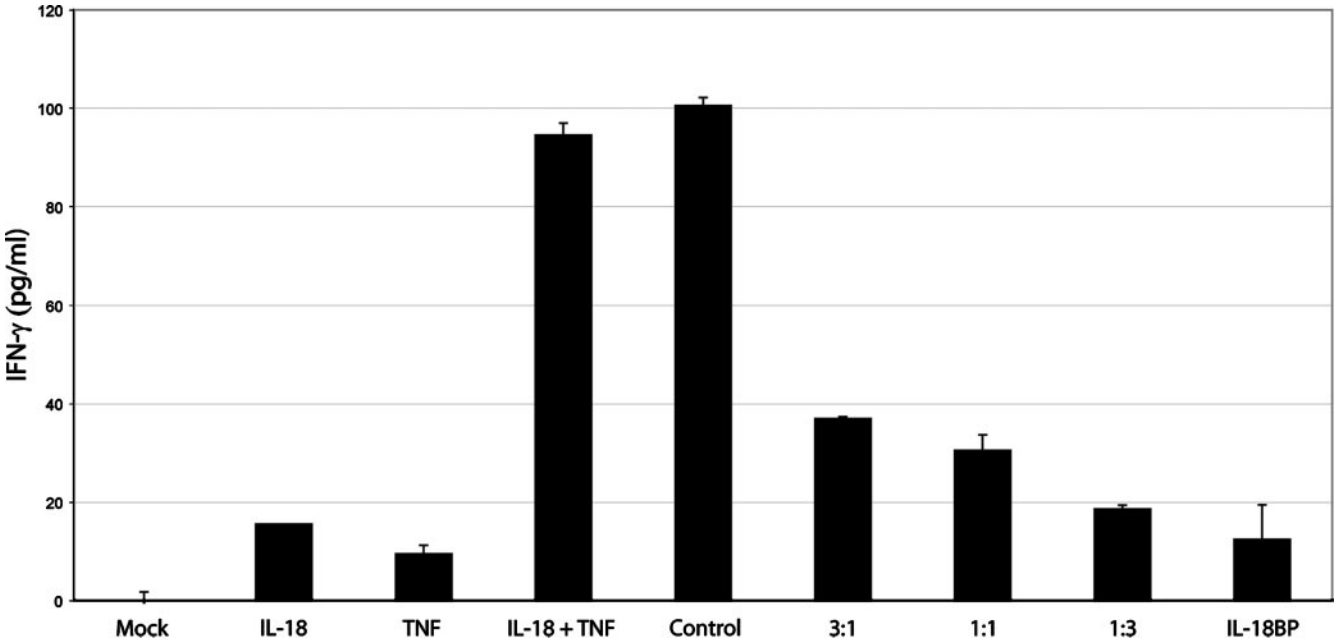

FIG. 3. Sequestration of hIL-18 with AcY14L-M/H. Protein A/G beads were incubated with anti-penta-His antibody and supernatants from insect cells infected with either AcY14L-M/H or AcNPVpolh^{-} (negative control). Beads were then mixed at the ratios (AcY14L-M/H/control) indicated below the sixth, seventh, and eighth bars, and hIL-18 (100 ng/ml) was added to the beads. TNF (5 ng/ml) and supernatants at a 1 in 10 dilution were added to KG-1 cells. IFN-γ was assayed by ELISA. Error bars show standard deviations.

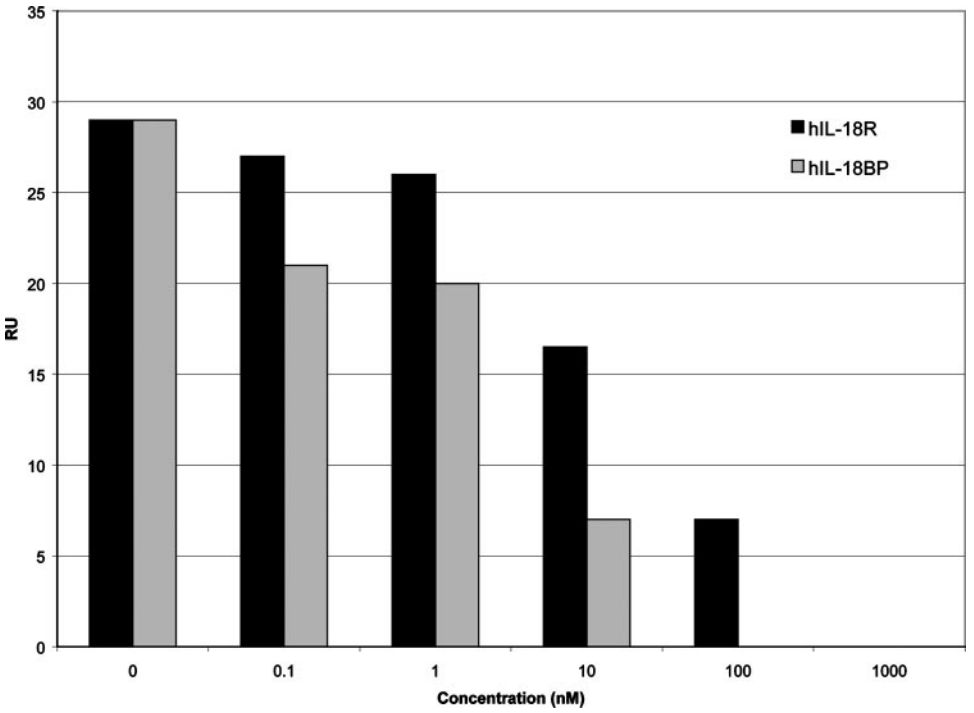

FIG. 4. The IL-18 binding site for YMTV IL-18BP overlaps with both hIL-18BP and hIL-18Rα. YMTV 14L was immobilized to a CM5 chip, 100 nM hIL-18 was incubated with the indicated concentrations of either hIL-18BP or hIL-18Rα for 30 min, and the solution was then injected over the sensor chip surface. The maximum level of binding is shown in relative units (RU).

R104A) are located on residues within site II (Fig. 5). Additionally, M60A, which is also located on a residue in site II, seems to effect a significant but less-dramatic decrease in affinity. The remaining mutations (R13A, D17A, and M33A) mapped to a compact cluster in site I (Fig. 5). Thus, the IL-18 domains critical for interaction with YMTV 14L are more delocalized on the cytokine surface than the sites determined to be critical for binding to other poxvirus IL-18BPs (13) (Fig. 6).

DISCUSSION

One of the ways poxviruses are able to subvert the host immune system is by encoding multiple virulence factors that

TABLE 2. Kinetics and affinity constants of hIL-18 mutants binding to YMTV 14L[a]

hIL-18	K_a (10^5/M · s)	K_d (10^{-4}/s)	K_D (nM)
Wild type	6.4 ± 0.1	1.0 ± 0.3	0.16 ± 0.05
K4A mutant	3.6 ± 0.1	1.1 ± 0.4	0.30 ± 0.11
L5A mutant	4.2 ± 0.1	3.9 ± 0.3	0.94 ± 0.07
E6A mutant	12.1 ± 0.4	1.9 ± 0.3	0.16 ± 0.02
K8A mutant	11 ± 1.5	2.3 ± 0.3	0.21 ± 0.01
R13A mutant	5.8 ± 0.4	3.7 ± 0.1	0.64 ± 0.05
D17A mutant	3.1 ± 0.1	1.9 ± 0.4	0.62 ± 0.13
M33A mutant	4.8 ± 0.1	2.2 ± 0.3	0.44 ± 0.05
D35A mutant	12.5 ± 0.5	3.1 ± 0.2	0.24 ± 0.03
K53A mutant	4.4 ± 0.3	7.6 ± 0.5	1.73 ± 0.24
S55A mutant	2.3 ± 0.1	2.8 ± 0.6	1.24 ± 0.28
R58A mutant	3.1 ± 0.3	5.2 ± 0.6	1.71 ± 0.27
M60A mutant	6.0 ± 0.3	3.0 ± 0.2	0.51 ± 0.02
K79A mutant	7.1 ± 0.1	1.9 ± 0.4	0.27 ± 0.06
K84A mutant	18 ± 1.8	2.7 ± 0.7	0.15 ± 0.03
D98A mutant	23 ± 8.3	2.7 ± 0.3	0.13 ± 0.05
R104A mutant	1.8 ± 0.1	2.2 ± 0.4	1.23 ± 0.15
D132A mutant	18.4 ± 0.4	3.0 ± 0.4	0.16 ± 0.02

[a] Values are the means ± standard deviations of the results. K_a, association rate constant; K_d, dissociation rate constant; K_D, dissociation rate.

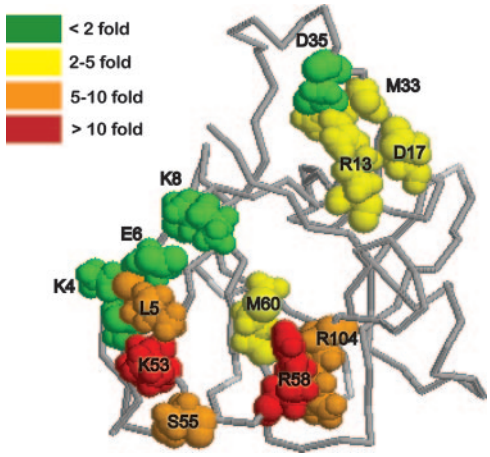

FIG. 5. YMTV 14L binding is influenced by several residues located on one face of hIL-18. Mutated residues are displayed in space-fill. Residues are colored based on the decrease (*n*-fold) in affinity of the mutant compared to that of wild-type hIL-18. Mutations R13A, D17A, D35A, and M33A are located on residues in site I; all other residues shown belong to site II. Residues in site III are not shown.

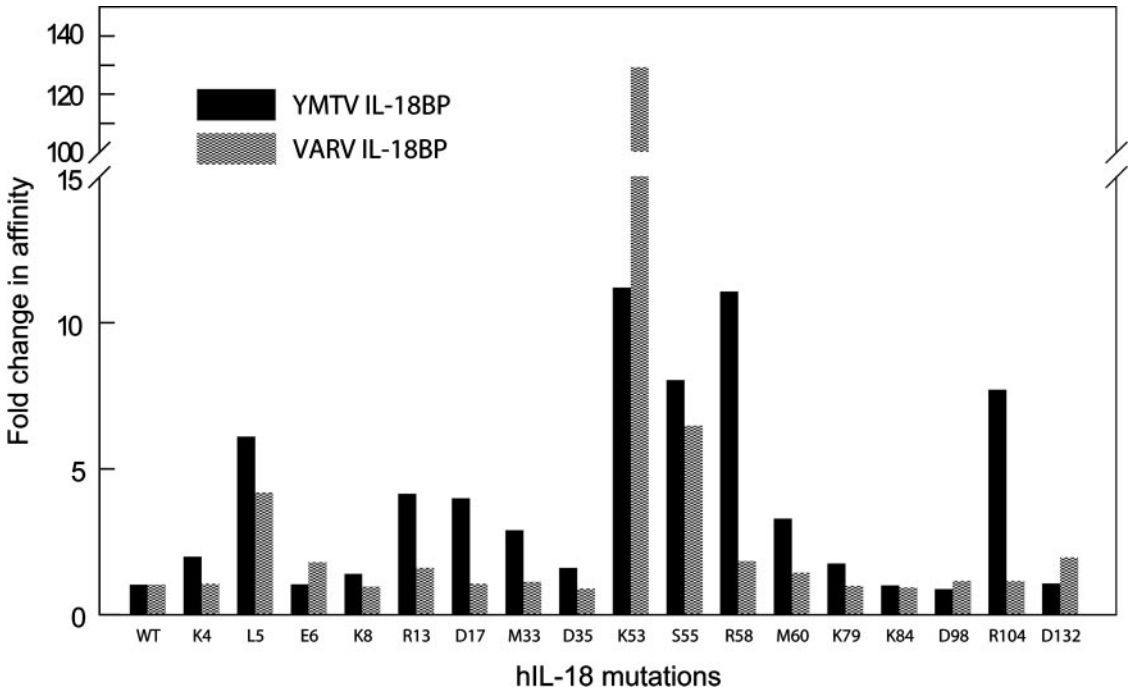

FIG. 6. YMTV 14L binds to hIL-18 in a more promiscuous manner than the VARV IL-18BP. Values for the graph were taken from reference 13 and from the current study. The change (*n*-fold) with respect to the affinity of the wild-type IL-18 is shown.

systematically inhibit the expression or biological properties of key secreted immune signaling molecules. Studies of these viral genes has suggested that many were likely once acquired as inhibitory regulators from an infected host, possibly as a recombined cDNA, and many of these viral immunomodulators exhibit inhibitory properties that are similar to those of their host homologues. Here, we characterize the YMTV IL-18BP protein, which is encoded by the 14L open reading frame of the YMTV genome, as binding and inhibiting hIL-18; however, our data on the altered binding properties suggest that it functions more to sequester the host cytokine than to directly inhibit IL-18 signaling through its cognate receptor, as is the case for traditional IL-18BPs.

In contrast to previously characterized poxviral IL-18BPs, YMTV 14L inhibits the biological signaling properties of IL-18 incompletely, despite the fact that it binds quantitatively to the cytokine with high affinity (Table 1; Fig. 3), similar to other poxviral IL-18BPs, and the fact that the binding site overlaps with that of IL-18Rα (Fig. 4). This can likely be attributed to the modified binding specificity compared to the specificities of the key contact residues of other poxviral IL-18BPs (i.e., VARV IL-18BP). Mutations of residues within both sites I and II of hIL-18 indicate that both sites are involved in binding to YMTV 14L. Unlike the results for the VARV IL-18BP, no single IL-18 mutation caused a dramatic decrease in affinity; however, many mutations significantly affected IL-18 binding.

This apparent delocalization of the IL-18 binding domain has led to a modification of 14L protein function since, while the YMTV IL-18BP still has a high affinity for IL-18 as measured by binding and sequestration assays, it is unable to fully inhibit hIL-18's biological activity in an IL-18-dependent IFN-γ release assay. This functional aspect of the 14L protein is not due to an inability to bind tightly to hIL-18 under the assay conditions, since the YMTV IL-18BP is able to fully sequester all active hIL-18 under the same conditions. This suggests that the mechanism of action has possibly evolved to prevent IL-18 from reaching its target cellular receptors rather than as a classical inhibitory complex that prevents receptor signaling.

A detailed study of IL-18BP evolution was recently published in which the authors examined the phylogenetic ancestry of 24 IL-18BP family members, including 13 from chordopoxviruses (22). Interestingly, many poxviral IL-18BPs have nonconservative mutations in residues identified as critical for binding to IL-18, including the MOCV IL-18BP, a functional inhibitor of hIL-18 (22, 24, 25). The authors of the study also hypothesize that the acquisition of the IL-18BP gene occurred in two separate events; the first event occurred in an ancestor of MOCV and the orthopoxviruses, while the second event occurred in an ancestor of several poxviruses, including the capripoxviruses, *Swinepox virus*, and YMTV (22). This predicted, independent acquisition of an IL-18BP by a separate branch of chordopoxviruses may help to explain the biochemical differences observed among the IL-18BPs. Since the gene may have been acquired separately by YMTV and therefore been under different selection pressures, it may not be surprising that its mode of action has diverged from those of the orthologs described for the orthopoxvirus IL-18BP, MOCV IL-18BP, and hIL-18BP. Importantly, the IL-18BPs from the *Capripoxviridae* and *Swinepox virus* have yet not been characterized. Comparisons between the YMTV IL-18BP and those of other poxviruses that are thought to have acquired the gene in the same acquisition event should be highly informative.

The increased promiscuity and altered IL-18 inhibition pro-

file of YMTV 14L may indicate a slightly different function for the YMTV 14L in vivo than those of the other characterized poxviral IL-18BPs. The function of 14L in the virulence of YMTV has not been tested in nonhuman primates or humans, but it is unlikely that the divergence of 14L would result in a poorly functioning protein.

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REFERENCES

1. **Born, T. L., L. A. Morrison, D. J. Esteban, T. VandenBos, L. G. Thebeau, N. Chen, M. K. Spriggs, J. E. Sims, and R. M. Buller.** 2000. A poxvirus protein that binds to and inactivates IL-18, and inhibits NK cell response. J. Immunol. **164:**3246–3254.
2. **Brunetti, C. R., H. Amano, Y. Ueda, J. Qin, T. Miyamura, T. Suzuki, X. Li, J. W. Barrett, and G. McFadden.** 2003. Complete genomic sequence and comparative analysis of the tumorigenic poxvirus Yaba monkey tumor virus. J. Virol. **77:**13335–13347.
3. **Calderara, S., Y. Xiang, and B. Moss.** 2001. Orthopoxvirus IL-18 binding proteins: affinities and antagonist activities. Virology **279:**22–26.
4. **Dinarello, C. A.** 1999. IL-18: a TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J. Allergy Clin. Immunol. **103:**11–24.
5. **Dinarello, C. A.** 2007. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin. Nephrol. **27:**98–114.
6. **Dinarello, C. A., and G. Fantuzzi.** 2003. Interleukin-18 and host defense against infection. J. Infect. Dis. **187**(Suppl. 2)**:**S370–S384.
7. **Downie, A. W., and C. Espana.** 1972. Comparison of Tanapox virus and Yaba-like viruses causing epidemic disease in monkeys. J. Hyg. (London) **70:**23–32.
8. **Ghayur, T., S. Banerjee, M. Hugunin, D. Butler, L. Herzog, A. Carter, L. Quintal, L. Sekut, R. Talanian, M. Paskind, W. Wong, R. Kamen, D. Tracey, and H. Allen.** 1997. Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. Nature **386:**619–623.
9. **Johnsson, B., S. Lofas, and G. Lindquist.** 1991. Immobilization of proteins to a carboxymethyldextran-modified gold surface for biospecific interaction analysis in surface plasmon resonance sensors. Anal. Biochem. **198:**268–277.
10. **Kato, Z., J. Jee, H. Shikano, M. Mishima, I. Ohki, H. Ohnishi, A. Li, K. Hashimoto, E. Matsukuma, K. Omoya, Y. Yamamoto, T. Yoneda, T. Hara, N. Kondo, and M. Shirakawa.** 2003. The structure and binding mode of interleukin-18. Nat. Struct. Biol. **10:**966–971.
11. **Kim, S. H., M. Eisenstein, L. Reznikov, G. Fantuzzi, D. Novick, M. Rubinstein, and C. A. Dinarello.** 2000. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc. Natl. Acad. Sci. USA **97:**1190–1195.
12. **Knight, J. C., F. J. Novembre, D. R. Brown, C. S. Goldsmith, and J. J. Esposito.** 1989. Studies on Tanapox virus. Virology **172:**116–124.
13. **Meng, X., M. Leman, and Y. Xiang.** 2007. Variola virus IL-18 binding protein interacts with three human IL-18 residues that are part of a binding site for human IL-18 receptor alpha subunit. Virology **358:**211–220.
14. **Nazarian, S. H., and G. McFadden.** 2006. Immune evasion by poxviruses. Future Virol. **1:**123–132.
15. **Novick, D., S. H. Kim, G. Fantuzzi, L. L. Reznikov, C. A. Dinarello, and M. Rubinstein.** 1999. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. Immunity **10:**127–136.
16. **Petrilli, V., S. Papin, and J. Tschopp.** 2005. The inflammasome. Curr. Biol. **15:**R581.
17. **Puren, A. J., G. Fantuzzi, and C. A. Dinarello.** 1999. Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1beta are differentially regulated in human blood mononuclear cells and mouse spleen cells. Proc. Natl. Acad. Sci. USA **96:**2256–2261.
18. **Smith, V. P., N. A. Bryant, and A. Alcami.** 2000. Ectromelia, vaccinia and cowpox viruses encode secreted interleukin-18-binding proteins. J. Gen. Virol. **81:**1223–1230.
19. **Suzuki, N., N. J. Chen, D. G. Millar, S. Suzuki, T. Horacek, H. Hara, D. Bouchard, K. Nakanishi, J. M. Penninger, P. S. Ohashi, and W. C. Yeh.** 2003. IL-1 receptor-associated kinase 4 is essential for IL-18-mediated NK and Th1 cell responses. J. Immunol. **170:**4031–4035.
20. **Symons, J. A., E. Adams, D. C. Tscharke, P. C. Reading, H. Waldmann, and G. L. Smith.** 2002. The vaccinia virus C12L protein inhibits mouse IL-18 and promotes virus virulence in the murine intranasal model. J. Gen. Virol. **83:**2833–2844.
21. **Takeda, K., H. Tsutsui, T. Yoshimoto, O. Adachi, N. Yoshida, T. Kishimoto, H. Okamura, K. Nakanishi, and S. Akira.** 1998. Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity **8:**383–390.
22. **Watanabe, M., N. Goto, Y. Watanabe, S. Nishiguchi, K. Shimada, T. Yasunga, and H. Yamanishi.** 2005. Evolution of interleukin-18 binding proteins and interleukin-1 receptor, type II proteins. Int. J. Mol. Med. **15:**561–566.
23. **Xiang, Y., and B. Moss.** 2001. Correspondence of the functional epitopes of poxvirus and human interleukin-18-binding proteins. J. Virol. **75:**9947–9954.
24. **Xiang, Y., and B. Moss.** 2001. Determination of the functional epitopes of human interleukin-18-binding protein by site-directed mutagenesis. J. Biol. Chem. **276:**17380–17386.
25. **Xiang, Y., and B. Moss.** 1999. IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. Proc. Natl. Acad. Sci. USA **96:**11537–11542.
26. **Yoo, J. K., H. Kwon, L. Y. Khil, L. Zhang, H. S. Jun, and J. W. Yoon.** 2005. IL-18 induces monocyte chemotactic protein-1 production in macrophages through the phosphatidylinositol 3-kinase/Akt and MEK/ERK1/2 pathways. J. Immunol. **175:**8280–8286.